(12) UK Patent Application (19) GB (11) 2 089 823 A

(21) Application No 8135734
(22) Date of filing
26 Nov 1981
(30) Priority data
(31) 80/41181
(32) 23 Dec 1980
(33) United Kingdom (GB)
(43) Application published
30 Jun 1982
(51) INT CL³ C08G 63/72
(52) Domestic classification
C3R 37N2 37PX 37T1X
LB
(56) Documents cited
None
(58) Field of search
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(54) **Extraction of poly(β-hydroxybutyric acid)**

(57) Poly(β-hydroxybutyric acid), PHB, extraction by agglomeration of spray dried bacterial cells containing PHB with flocculated cells, or with some cell suspension, followed by contact with a solvent.

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SPECIFICATION

(54) Extraction of poly(β-hydroxybutyric acid)

This invention relates to the extraction of poly(β-hydroxybutyric acid) hereinafter referred to as PHB.

PHB is a thermoplastic polyester that is useful as a plastics material. It is accumulated by many bacteria as an energy reserve material in the form of granules within the bacterial cells.

While bacterial cells containing PHB can be used as such as a moulding material, for example as described in PSP 3,107,172, it is generally desirable to separate the PHB from the remainder of the bacterial cell material.

One method that has been proposed for the extraction of PHB from the suspension of cells produced by culturing the bacteria in an aqueous medium on a suitable carbon and energy source is to remove the water from the suspension, eg by spray or flash drying, followed by contacting the dried cells with a solvent in which PHB is soluble (see European Patent Application 15123).

The spray or flash drying process effects sufficient breakage of the bacterial cell to permit the PHB contained therein to be extracted. However one disadvantage of this process is that it is necessary to separate the PHB-containing solution from the cell debris. Because of the small size of the bacterial cells, and hence of the fragments resulting therefrom, techniques such as filtration have heretofore presented difficulties. This is particularly true where the PHB-containing solution is relatively viscous as is the case where chloroform is utilised as the solvent.

We have found that, if the dried cell powder is agglomerated, the PHB can be leached out leaving the residual cell material still agglomerated and hence more readily separable from the solution of the extracted PHB in the solvent therefor.

Suitable agglomerates may be made by mixing the dried cells, eg by tumble blending, with a small amount of a bacterial cell suspension or bacterial cell flocs.

Accordingly we provide a process for the extraction of PHB from an aqueous suspension of PHB-containing bacterial cell comprising

(i) forming dried bacterial cells by introducing said suspension in finely divided form into a current of gas heated to a temperature of at least 100°C to evaporate the water from the suspension,

(ii) forming particulate agglomerates of said dried cells by mixing said dried cells with a sufficient quantity of bacterial cells in the form of

(a) an aqueous cell suspension, or

(b) flocs formed by causing the cells in an

to bind said dried cells together,

(iii) extracting PHB from said agglomerates by contacting the agglomerates with a solvent for PHB, and

(iv) separating the solvent having the PHB dissolved therein from the residual cell material.

The dried cells are produced by a drying process involving introducing the cell suspension in finely divided form, eg as a spray or fine stream into a current of gas, eg air, heated to a temperature of at least 100°C. Preferably the suspension is introduced via a spray or atomising nozzle. Such drying processes are well known and include spray and flash drying.

The heated gas current evaporates off the water which is carried away by the gas stream leaving the dried cells which are collected for extraction with the PHB extraction solvent.

The gas inlet temperature may be in the range 100°C to 300°C and is preferably in the range 120°C to 250°C.

The bacteria whose cells are used to bind the dried PHB-containing cells together may be the same as, or different from, the bacteria used to produce the dried PHB-containing cells. For convenience however it is preferred that the dried cells are bound into the agglomerates by means of a small quantity of the same suspension that is fed to the gas stream or by flocs produced from such a suspension.

Flocs may be produced from a cell suspension by reducing the pH of the suspension to a value within the range 2 to 5 by treatment with an acid, the suspension being treated with an alkali to increase its pH to a value in the range 8 to 12 before acidification and/or heated to a temperature within the range 50 to 200°C before or after acidification. Examples of such flocculation processes are described in UK Patent Specifications 1,062,005 and 1,381,306. Preferably the suspension is flocculated by increasing its pH to a value in the range 10 to 12, heating by injecting steam under pressure into the cell suspension, and then acidifying to a pH in the range 3 to 5. The amount and temperature of the steam is preferably such as to raise the temperature of the cell suspension to a temperature in the range 60 to 100°C. The flocculated cells may be separated from the aqueous medium by filtration, sedimentation, flotation, or centrifugation.

The amount of the bacterial cells used as the binder should preferably be sufficient to bind the dried cells into suitably sized agglomerates without rendering the latter sticky. The agglomerates preferably have an average size within the range 0.3 to 2mm. The agglomerates may be made by tumble blending the dried cells with the binder cells. Simple experimentation will determine the requisite proportion of binder cells. Where the binder cells are in the form of a cell suspension, the quantity

of binder cells is preferably 15 to 60% by weight of the dried cells and the suspension has a biomass content of 50 to 200 g l^{-1}. Where flocs are employed as the binder cells, the amount thereof is preferably 10 to 40% by weight of the dried cells.

Inert particulate fillers may also be incorporated into the agglomerates, eg to increase the porosity thereof. Examples of such inert fillers include alumina.

Where the binder cells contain PHB, little of the PHB therein will be extracted when the binder is in the form of a cell suspension since little or no breakage of the binder cells takes place during the tumbling and extraction steps. Hence if it is desired to recover the PHB in the binder cells, it may be desirable to subject the cell suspension to a preliminary cell breakage step such as milling.

Some cell breakage does occur during flocculation and so, where the binder cells contain PHB, it is preferred, for optimum PHB recovery, to use the binder cells in the form of flocs. On the other hand the cost of the flocculation step may render it more economic to use a cell suspension as the binder and to forego recovery of the PHB within the binder cells.

After forming the agglomerates, the PHB in the dried cells in the agglomerates is extracted by contacting the agglomerate with a solvent in which PHB is soluble.

Before being contacted with the PHB-extraction solvent, the agglomerated cells are preferably contacted with a solvent in which the lipids associated with the bacterial cell are soluble but in which PHB is insoluble. Examples of such solvents include methanol and acetone. The extraction of lipids is preferably effected at elevated temperatures, eg 40 to 90°C, although sufficient lipid extraction may be effected in some cases using lower temperatures, eg 25 to 40°C. The use of the elevated temperatures is preferred as, in general, when using such elevated temperatures, the agglomerates tend to sink in the lipid extraction solvent thus rendering separation of the agglomerates and lipid extraction solvent facile by techniques such as decanting.

The cells are then contacted with the PHB-extraction solvent. Preferred extraction solvents include 1,2-dichloroethane, methylene chloride and cloroform. Where no preliminary lipid extraction step is employed, the PHB extraction is preferably effected at tempertures below 40°C, whereas higher temperatures, eg 50 to 90°C may advantageously be employed if a preliminary lipid extraction step is utilised.

The lipid extraction step (if used) and/or the PHB extraction may be effected continuously with the agglomerates packed into a suitable bed.

We have found that the PHB may readily be leached from the agglomerates leaving the cell residue in agglomerate form. This cell residue can be separated from the PHB-containing solution easily by techniques such as filtration. The agglomerated dried cells are particularly suited to trickle extraction techniques wherein the PHB-solvent is allowed to permeate down through a bed of the agglomerates.

The PHB may be recovered from the solution in the extraction solvent by precipitation into a non-solvent, eg a methanol/water mixture or by evaporation of the solvent, eg by spray or flash drying.

The invention is illustrated by the following example.

The major portion of an aqueous suspension of *Alcaligenes eutrophus* of 150 g l^{-1} biomass content, of which about 45% by weight was PHB, was spray dried at a suspension feed rate of 5000ml hr^{-1}, an air inlet temperature of 240°C, an air outlet temperature of 110°C, and an air flow rate of 300 m^3 hr^{-1}. The resultant powder consisting of the dried cells had a size below 150μm.

100g of the spray dried cells were granulated by mixing with 47 ml of the original aqueous suspension in a laboratory mixer for 5 min at 60°C. The granules were dried in a fluidised bed for 25 min using an air inlet temperature of 100°C.

10g of the dried granules were refluxed with 200 ml of chloroform for 5 min to extract PHB. The cell debris was granular and floated on top of the chloroform solution and was readily skimmed therefrom.

The PHB was then precipitated from the chloroform solution by adding the solution to 800 ml of a mixture of methanol and water (4 vol. methanol : 1 vol. water). The precipitated PHB was recovered by filtration and dried in an oven at 40°C.

The weight of PHB recovered was about 2.7g.

The weight average molecular weight of the recovered PHB was 205,000 as measured by gel permeation chromatography.

By way of comparison, when the spray dried cells were extracted directly by refluxing with chloroform, the cell debris was in the form of fine particles that could only be separated from the chloroform solution with difficulty.

CLAIMS

1. A process for the extraction of PHB from an aqueous suspension of poly(β-hydroxybutyric acid), (PHB), containing bacterial cells comprising

(i) forming dried bacterial cells by introducing said suspension in finely divided form into a current of gas heated to a temperature of at least 100°C to evaporate the water from the suspension,

(ii) forming particulate agglomerates of said dried cells by mixing said dried cells with a sufficient quantity of bacterial cells in the form

of

(a) an aqueous cell suspension, or

(b) flocs formed by causing the cells in an aqueous cell suspension to flocculate,

to bind said dried cells together,

(iii) extracting PHB from said agglomerates by contacting the agglomerates with a solvent for PHB, and

(iv) separating the solvent having the PHB dissolved therein from the residual cell material.

2. A process according to claim 1 wherein the bacterial cells used to bind the dried cells together are said PHB-containing bacterial cells.

3. A process according to claim 1 or claim 2 wherein said agglomerates have an average size within the range 0.3 to 2 mm.

4. A process according to any one of claims 1 to 3 wherein a bacterial cell suspension containing 50 to 200 $g.1^{-1}$ of biomass is used to bind said dried cells together, the amount of said bacterial cell suspension being such that the weight of cells therein is 15 to 60% by weight of the dried cells.

5. A process according to any one of claims 1 to 3 wherein said dried cells are bound together by 10 to 40% by weight, based on the weight of said dried cells, of flocculated cells.

6. A process according to any one of claims 1 to 5 wherein, prior to contact with said solvent, the agglomerated cells are contacted with a solvent in which PHB is insoluble but in which lipids associated with the bacterial cells are soluble.

7. A process according to claim 6 wherein said agglomerated cells are contacted with said solvent in which said lipids are soluble but in which PHB is insoluble at a temperature in the range 40 to 90°C.

8. A process according to claim 6 or claim 7 wherein the agglomerated cells are contacted with the solvent in which the PHB is soluble at a temperature in the range 50 to 90°C.

Printed for Her Majesty's Stationery Office
by Burgess & Son (Abingdon) Ltd.—1982.
Published at The Patent Office, 25 Southampton Buildings,
London, WC2A 1AY, from which copies may be obtained.